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## POSITION STATEMENT

# Cancer Variant Interpretation Group UK (CanVIG-UK): an exemplar national subspecialty multidisciplinary network

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## ABSTRACT

Advances in technology have led to a massive expansion in the capacity for genomic analysis, with a commensurate fall in costs. The clinical indications for genomic testing have evolved markedly; the volume of clinical sequencing has increased dramatically; and the range of clinical professionals involved in the process has broadened. There is general acceptance that our early dichotomous paradigms of variants being pathogenic–high risk and benign–no risk are overly simplistic. There is increasing recognition that the clinical interpretation of genomic data requires significant expertise in disease–gene–variant associations specific to each disease area. Inaccurate interpretation can lead to clinical mismanagement, inconsistent information within families and misdirection of resources. It is for this reason that ‘national subspecialist multidisciplinary meetings’ (MDMs) for genomic interpretation have been articulated as key for the new NHS Genomic Medicine Service, of which Cancer Variant Interpretation Group UK (CanVIG-UK) is an early exemplar. CanVIG-UK was established in 2017 and now has >100 UK members, including at least one clinical diagnostic scientist and one clinical cancer geneticist from each of the 25 regional molecular genetics laboratories of the UK and Ireland. Through CanVIG-UK, we have established national consensus around variant interpretation for cancer susceptibility genes via monthly national teleconferenced MDMs and collaborative data sharing using a secure online portal. We describe here the activities of CanVIG-UK, including exemplar outputs and feedback from the membership.

salpingo-oophorectomy and colectomy); (2) chemo-prevention; (3) intensive screening; and (4) lifestyle modification.<sup>2</sup> Family members negative for the familial CSG-PV can be spared anxiety and unnecessary screening. Many CSGs are associated with a pattern of cancer risk that is late-onset, variably penetrant and of autosomal dominant inheritance. PV-positive family members identified via cascade screening are often distributed across disparate genomics services.

Erroneous interpretation of CSG variant pathogenicity can therefore result in (1) discordant management within families, (2) serious clinical consequences for individuals and (3) misdirection at population level of resources for screening and prevention.<sup>3–5</sup> Increasingly, CSG-PVs are used as predictive biomarkers to inform cancer therapy. For all these reasons, robust, rapid, accurate variant analysis and interpretation of disease risk are critical to effective delivery of germline cancer genetics and improving outcomes for patients.

## Evolving landscape of variant interpretation in germline cancer genetics

In the late 1990s, within a few years of identification of the relevant genes, laboratory analysis of CSGs became available in the UK via family cancer clinics.<sup>2</sup> If the cancer phenotype ascribed to the gene matched that found in the proband/family under study, with little additional evidence, a rare variant would often be labelled as pathogenic and thus causative.<sup>6</sup> Subsequent large-scale population sequencing studies have revealed the degree of innocuous variation present in the human genome (and indeed in disease-associated genes) and ‘downgrading’ of many erroneously labelled PVs has been required.<sup>7</sup> An era of caution followed, with much greater recourse to labelling of variants as ‘variants of uncertain significance’ (VUS/VOUS). However, lack of systems for sharing new evidence has meant that many families have spent years in limbo with their ‘VUS’, even when data had long been available by which classification of their variant could be downgraded or upgraded.

## BACKGROUND

### Clinical utility of cancer susceptibility genes (CSGs)

Analysis of germline (constitutional) variants in CSGs constitutes approximately one-quarter of activity in NHS Molecular Diagnostic Laboratories in England.<sup>1</sup> Following identification of a pathogenic variant (PV) in a CSG, incidence of/mortality from future cancers may be mitigated via (1) risk-reducing surgery (eg, mastectomy, gastrectomy,



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Sharing of clinical variant data was somewhat improved with the advent of locus-specific databases (LSDs), such as Breast Cancer Information Core and Leiden Open Variant Databases.<sup>8–11</sup> However, the curation of clinical and molecular data in LSDs often remains suboptimal, with (1) erroneous nomenclature, (2) duplication of entries and (3) use of differing classification systems resulting in contradictory assignments.<sup>12</sup>

Using Myriad Genetics data from ~70 000 genetic tests for hereditary breast and ovarian cancer, in 2007, Easton and colleagues published a landmark multifactorial analysis through which ‘odds of causality’ were mathematically generated for 1433 variants using clinical, pedigree and allelic data.<sup>13</sup> In 2008, International Agency for Research on Cancer (IARC) collaborators published the first formal five point variant interpretation system for CSGs, which included numeric thresholds for the probability of pathogenicity.<sup>14</sup> Expert cancer susceptibility consortia such as the Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) and the International Society for Gastrointestinal Hereditary Tumours (InSIGHT) further evolved these multifactorial variant classification systems to incorporate tumour phenotype and in silico predictions.<sup>15–17</sup> However, ENIGMA/InSIGHT approaches require statistical genetic–epidemiological analyses of large curated data series and are not reproducible by an individual diagnostic laboratory seeking to classify in a clinically relevant timescale a newly identified variant.

In 2015, the American College of Medical Geneticists (ACMG) published a variant interpretation framework enabling the combination by a diagnostic laboratory of disparate evidence sources for a newly identified genomic variant.<sup>18</sup> The ACMG framework has subsequently been further evolved under the auspices of ClinGen, including (1) specification for how it is applied to particular genes and/or diseases (including *TP53*, *CDH1* and *PTEN*); (2) deeper specification of particular criteria (eg, functional assays); and (3) exposition of the underpinning Bayesian model.<sup>19–23</sup>

### Coordinated national UK approaches in variant interpretation

In 2016, with endorsement from NHS England and Health Education England, it was agreed formally by the UK Association of Clinical Genomic Science (UK-ACGS) to adopt the ACMG variant interpretation framework.<sup>24 25</sup> The UK-ACGS established national groups for rare disease, germline cancer genetics, cardiac disease and hypercholesterolaemia to develop and disseminate practice in the application of the ACMG variant interpretation framework.<sup>24</sup> In parallel was recognition within the NHS Genomic Medicine Service of the need for national subspecialist genomics MDMs.<sup>26 27</sup> In response to these dual recommendations, Cancer Variant Interpretation Group UK (CanVIG-UK) was initiated in 2017.

### CANCER VARIANT INTERPRETATION GROUP UK

The purpose of CanVIG-UK is to advance outcomes for patients by improving the accuracy and consistency of interpretation of variants in CSGs across the UK clinical genetics and molecular diagnostic laboratory communities (hereafter termed the UK clinical-laboratory community). We aim to progress this goal by advancing six objectives (see box 1).

#### Creation of a national multidisciplinary professional network and regular forum

CanVIG-UK has grown to now include >100 members, incorporating clinical and laboratory representation from each of the

### Box 1 CanVIG Objectives

The purpose of Cancer Variant Interpretation Group UK (CanVIG-UK) is to advance outcomes for patients by improving the accuracy and consistency of interpretation of variants in Cancer Susceptibility genes across the UK clinical-laboratory community. We have six specific objectives:

1. Creation of a national multidisciplinary professional network and regular forum.
2. Training and education.
3. Detailed specification for germline cancer genetics of the UK-ACGS Best Practice Guidelines for Variant Interpretation.
4. Ratification of additional guidance in germline cancer genetics relevant to the UK clinical-laboratory community.
5. Development of an online platform to facilitate information sharing and variant interpretation within the UK clinical-laboratory community.
6. UK contribution to international variant interpretation endeavours.

25 Molecular Diagnostic Laboratories and Clinical Genetics Services of the UK (NHS) and Ireland (see collaborators). This group comprises roughly equal proportions of clinical scientists and clinical geneticists, with two-thirds working exclusively or predominantly in cancer genetics (figure 1):

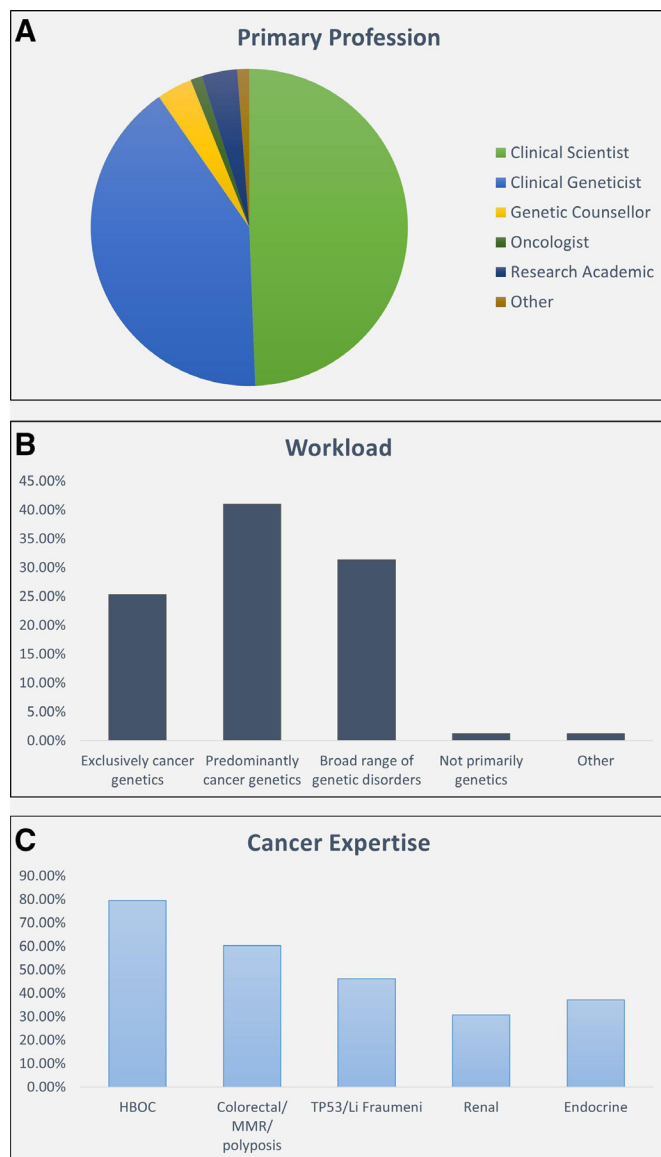
- The monthly teleconferenced MDM provides a *forum* to which problematic variants/cases are submitted. The variants submitted to the monthly variant surgery are circulated 1 week in advance. CanVIG-UK members are asked (1) to ascertain whether additional cases and/or laboratory data exist locally and (2) to undertake local, independent classification of the variant. The relevant clinical and laboratory data are presented by the nominating laboratory. This is followed by input of any additional information by the broader CanVIG-UK group and a discussion regarding the legitimacy of the ACMG criteria awarded. Following this discussion and an online postdiscussion poll, a consensus CanVIG classification is generated (see online supplementary table 1). A detailed date-stamped CanVIG variant summary sheet is generated (see online supplementary appendix 2), which is circulated by email, uploaded to the CanVar-UK portal and submitted to ClinVar.
- The CanVIG-UK *network* is active throughout the month via the email forum, through which urgent queries can be debated and addressed.

#### Training and education

The discussion of cases at the MDM also provides valuable education for the clinical-laboratory community regarding application of the ACMG framework and the vagaries of the evidence sources used (see figure 2). Additionally, through CanVIG-UK, we have supported training of the broader UK genetics and oncology communities in variant interpretation for CSGs.

#### Detailed specification for germline cancer genetics of the UK-ACGS Best Practice Guidelines for Variant Interpretation

On behalf of the UK-ACGS, the rare disease variant interpretation group has generated and updates annually a highly detailed specification of the ACMG variant interpretation framework.<sup>24</sup> In cancer susceptibility, we typically observe variants relating to late-onset, common phenotypes. De novo and biallelic



**Figure 1** Overview of the CanVIG-UK membership profile (survey of CanVIG-UK members, performed on 29 October 2019, return rate 83/103 (81%). HBOC, hereditary breast and ovarian cancer; MMR, mismatch repair. CanVIG-UK, Cancer Variant Interpretation Group UK.

paradigms are infrequent. We are typically much more reliant on variant frequency from case series and functional assays. Thus, an important remit for CanVIG-UK has been to develop a detailed specification of the UK-ACGS framework for these types of evidence to be used for CSG variant interpretation (see online supplementary appendix 1).

### Ratification of additional guidance in germline cancer genetics relevant to the UK clinical-laboratory community

Historically, the first presentation to the family cancer clinic was typically an unaffected individual, concerned by a significant family history. Increasingly, genetic analysis is now performed as part of routine work-up at cancer diagnosis, either through analysis of a germline sample or through therapeutically motivated molecular analysis of the tumour. In both contexts, (1) focused testing of one or two genes has often been superseded by broad 'cancer panels' containing dozens or hundreds of genes; (2) patients may be unselected for family history; and (3)

analysis and reporting in a tight time frame is typically required. A number of challenging issues have emerged, including

1. Categorisation and management of reduced penetrance variants in high-penetrance genes.
2. Variant interpretation and clinical management for moderate-penetrance genes.
3. Adaptation of variant interpretation and risk for different contexts of ascertainment.
4. Inference of germline findings from tumour-only sequencing.

While germane across genomics, consideration of these issues has become pressing within germline cancer genetics. Benefitting from its regular forum, multidisciplinary membership and alignment with both UK-ACGS and the UK Cancer Genetics Group (UK-CGG), we have used the CanVIG-UK monthly forum to evolve UK national multidisciplinary approaches on such issues (see online supplementary appendix 3).

### Development of an online platform to facilitate information sharing and variant interpretation within the UK clinical-laboratory community

In germline cancer genetics, enrichment in cases (especially 'strong families') is one of the most valuable clinical observations indicating variant pathogenicity. However, to date, we have struggled to quantify such observations on account of (1) failure to aggregate national data from distributed laboratories and (2) lack of a robust denominator.

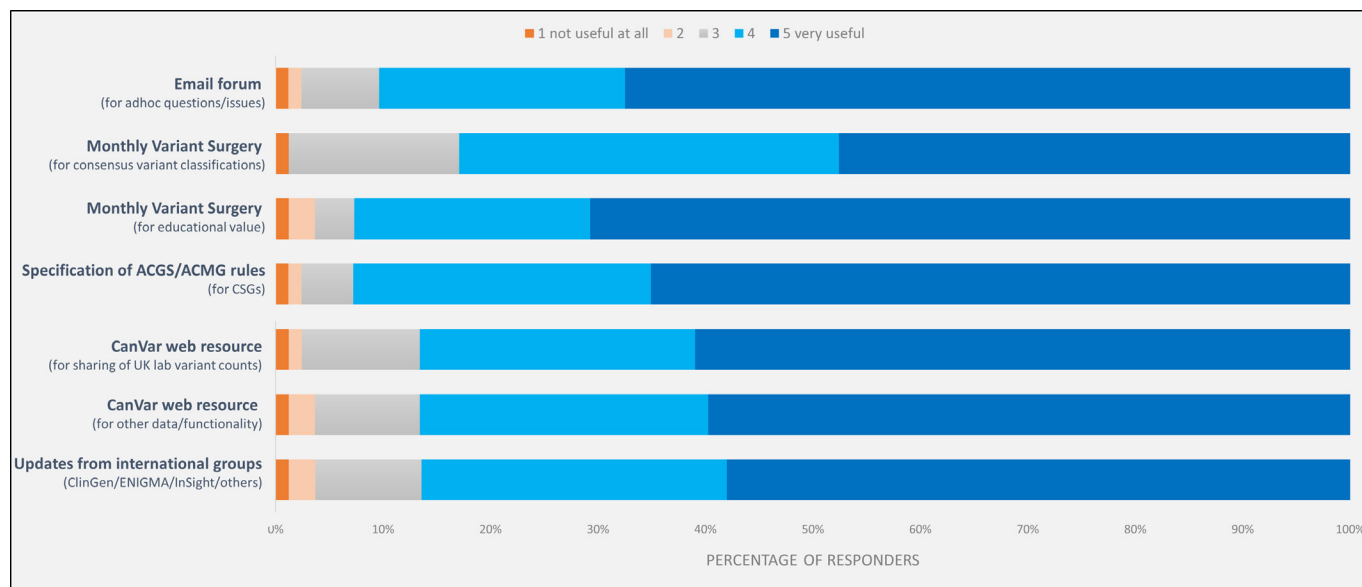
In a collaborative venture between Public Health England (PHE) and the national network of molecular diagnostic laboratories, data from molecular testing of CSGs have been submitted via a pseudonymisation portal to the secure National Cancer Registration and Analysis Service (NCRAS) data environment of PHE.<sup>28</sup> The national variant totals (numerator and denominator) are then shared by CanVIG-UK with the UK clinical-laboratory community via our online data system CanVar-UK (<http://www.canvaruk.org/>).

CanVar-UK provides additional annotations for 1008 643 variants from 95 CSGs. It includes variant-level annotations from LSDs (case counts), functional assays, splicing assays and multifactorial analyses for selected genes. Accessible only to registered CanVIG-UK clinical-laboratory users is a community area for sharing non-identifiable variant-level data, such as local classifications, comments/notes, uploaded documents and results from local laboratory assays (eg, RNA analyses of potential splicing variants).

### UK contribution to international variant interpretation endeavours

CanVIG-UK is an effective conduit between the UK clinical-laboratory germline cancer genetics community and relevant international variant interpretation endeavours in several regards:

- First, there is representation at the international ClinGen SVI group from the leadership of the UK-ACGS rare disease variant interpretation group. The regular crosstalk between leadership of the UK groups enables appraisal of the ClinGen SVI group of emerging analyses and activity within CanVIG-UK and the UK clinical-laboratory cancer genetics community.
- Second, multiple members of CanVIG-UK are members of gene-specific international endeavours such as ENIGMA, InSIGHT and ClinGen expert groups.
- Third, data generated by CanVIG-UK data have contributed to collaborative international consortia analyses, for



**Figure 2** Perceived utility of CanVIG-UK activities regarding local practice in CSG variant interpretation of seven activities (5: very useful to 1: not useful; survey of CanVIG-UK members, performed on 29 October 2019, return rate 83/103 (81%)). ACGS, Association of Clinical Genomic Science; ACMG, American College of Medical Geneticists; CanVIG-UK, Cancer Variant Interpretation Group UK; CSG, cancer susceptibility gene.

example, provision to ENIGMA of the summary PHE UK laboratory data on BRCA1/BRCA2 variants.

- Fourth, CanVIG-UK consensus classifications (and underpinning evidence) are shared via ClinVar. CanVIG-UK is the first UK organisation to submit clinical-laboratory variant classifications to ClinVar.

### Sustainability

Maintenance of a national multidisciplinary network, coordination of a regular teleconferenced MDMs and development of a data system is only feasible via sustained support. The activities of CanVIG-UK are currently supported by a Cancer Research UK Catalyst Award (CanGene-CanVar, @CangeneCanvar, C61296/A27223).

### CONCLUSION

CanVIG-UK is a multidisciplinary group comprising >100 clinical scientists and senior genetics clinicians working in germline cancer genetics, with representation from across the 25 NHS molecular diagnostic laboratories of the UK and Ireland. Through CanVIG-UK, the UK clinical-laboratory germline cancer genetics community have evolved:

1. An email forum for real-time consultation on problematic variants.
2. A monthly teleconferenced MDM for detailed review of challenging variants and cases.
3. A national programme of using secure submissions of frequency data from PHE.
4. An online data system (CanVar-UK) for sharing variant-level data both publicly and within a secure community region.
5. Detailed, consensus UK guidance for the interpretation of variants in CSGs.
6. Fruitful interactions with international CSG variant interpretation endeavours.

In summary, we propose CanVIG-UK as an exemplar National Subspecialty Multidisciplinary Genomics Network. In this era of rapid emergence of genomic knowledge, such networks are

becoming increasingly important to optimise collaborative specialist case review, information sharing and education.

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## REFERENCES

- Norbury G. Association of Clinical Genomic Science (ACGS) 2015-2016 Genetic Test Activity Audit 2017.
- Turnbull C, Sud A, Houlston RS. Cancer genetics, precision prevention and a call to action. *Nat Genet* 2018;50:1212–8.
- Tandy-Connor S, Gultinan J, Krempely K, LaDuca H, Reineke P, Gutierrez S, Gray P, Tippin Davis B. False-Positive results released by direct-to-consumer genetic tests highlight the importance of clinical confirmation testing for appropriate patient care. *Genet Med* 2018;20:1515–21.
- Kilbride MK, Domchek SM, Bradbury AR. Ethical implications of direct-to-consumer hereditary cancer tests. *JAMA Oncol* 2018;4:1327–8.
- The Guardian, 2019. Available: <https://www.theguardian.com/science/2019/jul/21/senior-doctors-call-for-crackdown-on-home-genetic-testing-kits>
- Eccles DM, Mitchell G, Monteiro ANA, Schmutzler R, Couch FJ, Spurdle AB, Gómez-García EB, ENIGMA Clinical Working Group. Brca1 and BRCA2 genetic testing-pitfalls and recommendations for managing variants of uncertain clinical significance. *Ann Oncol* 2015;26:2057–65.
- Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won H-H, Yu D, Altshuler DM, Ardisson D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG, Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016;536:285–91.
- Pinard A, Salgado D, Desvignes J-P, Rai G, Hanna N, Arnaud P, Guieu C, Martinez M, Faivre L, Jondeau G, Boileau C, Zaffran S, Bérout C, Collod-Bérout G. WES/WGS Reporting of Mutations from Cardiovascular "Actionable" Genes in Clinical Practice: A Key Role for UMD Knowledgebases in the Era of Big Databases. *Hum Mutat* 2016;37:1308–17.
- Pinard A, Miltgen M, Blanchard A, Mathieu H, Desvignes J-P, Salgado D, Fabre A, Arnaud P, Barré L, Krahn M, Grandval P, Olschwang S, Zaffran S, Boileau C, Bérout C, Collod-Bérout G. Actionable genes, core databases, and locus-specific databases. *Hum Mutat* 2016;37:1299–307.
- Szabo C, Masiello A, Ryan JF, Brody LC. The breast cancer information core: database design, structure, and scope. *Hum Mutat* 2000;16:123–31.
- Fokkema IFAC, Taschner GPC, Celi J, Laros JFJ, den Dunnen JT. LOVD v.2.0: the next generation in gene variant databases. *Hum Mutat* 2011;32:557–63.
- Greenblatt MS, Brody LC, Foulkes WD, Genuardi M, Hofstra RMW, Olivier M, Plon SE, Sijmons RH, Sinilnikova O, Spurdle AB, IARC Unclassified Genetic Variants Working Group. Locus-specific databases and recommendations to strengthen their contribution to the classification of variants in cancer susceptibility genes. *Hum Mutat* 2008;29:1273–81.
- Easton DF, Deffenbaugh AM, Pruss D, Frye C, Wenstrup RJ, Allen-Brady K, Tavtigian SV, Monteiro ANA, Iversen ES, Couch FJ, Goldgar DE. A systematic genetic assessment of 1,433 sequence variants of unknown clinical significance in the BRCA1 and BRCA2 breast cancer-predisposition genes. *Am J Hum Genet* 2007;81:873–83.
- Plon SE, Eccles DM, Easton D, Foulkes WD, Genuardi M, Greenblatt MS, Hogervorst FBL, Hoogerbrugge N, Spurdle AB, Tavtigian SV, IARC Unclassified Genetic Variants Working Group. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. *Hum Mutat* 2008;29:1282–91.
- Spurdle AB, Healey S, Devereau A, Hogervorst FBL, Monteiro ANA, Nathanson KL, Radice P, Stoppa-Lyonnet D, Tavtigian S, Wappenschmidt B, Couch FJ, Goldgar DE, ENIGMA. ENIGMA-evidence-based network for the interpretation of germline mutant alleles: an international initiative to evaluate risk and clinical significance associated with sequence variation in BRCA1 and BRCA2 genes. *Hum Mutat* 2012;33:2–7.
- Parsons MT, Tudini E, Li H, Hahnen E, Wappenschmidt B, Feliubadaló L, Aalfs CM, Agata S, Aittomäki K, Alducci E, Alonso-Cerezo MC, Arnold N, Auber B, Austin R, Azzollini J, Balmaña J, Barbieri E, Bartram CR, Blanco A, Blümcke B, Bonache S, Bonanni B, Borg Åke, Bortesi B, Brunet J, Bruzzzone C, Bucksch K, Cagnoli G, Caldes T, Caliebe A, Caligo MA, Calvello M, Capone GL, Caputo SM, Carnevali I, Carrasco E, Caux-Moncoutier V, Cavalli P, Cini G, Clarke EM, Concolino P, Cops EJ, Cortesi L, Couch FJ, Darder E, de la Hoya M, Dean M, Debatin I, Del Valle J, Delnatte C, Derive N, Diez O, Ditsch N, Domchek SM, Dutranoy V, Eccles DM, Ehrencrona H, Enders U, Evans DG, Farra C, Faust U, Felber U, Feroce I, Fine M, Foulkes WD, Galvao HCR, Gambino G, Gehrig A, Gensini F, Gerdes A-M, Germani A, Giesecke J, Gismondi V, Gómez C, Gómez García EB, González S, Grau E, Grill S, Gross E, Guerrieri-Gonzaga A, Guillaud-Bataille M, Gutiérrez-Enríquez S, Haaf T, Hackmann K, Hansen TVO, Harris M, Hauke J, Heinrich T, Hellebrand H, Herold KN, Honisch E, Horvath J, Houdayer C, Hübner V, Iglesias S, Izquierdo A, James PA, Janssen LAM, Jeschke U, Kaulfuß S, Keupp K, Kiechle M, Kölbl A, Krieger S, Kruse TA, Kvist A, Laloo F, Larsen M, Lattimore VL, Lautrup C, Ledig S, Leinert E, Lewis AL, Lim J, Loeffler M, López-Fernández A, Lucci-Cordisco E, Maass N, Manoukian S, Marabelli M, Matricardi L, Meindl A, Michelli RD, Moghadas S, Moles-Fernández A, Montagna M, Montalban G, Monteiro AN, Montes E, Mori L, Moserle L, Müller CR, Mundhenke C, Naldi N, Nathanson KL, Navarro M, Nevanlinna H, Nichols CB, Niederacher D, Nielsen HR, Ong K-R, Pachter N, Palmero EI, Papi L, Pedersen IS, Peissel B, Perez-Segura P, Pfeifer K, Pineda M, Pohl-Rescigno E, Poplawski NK, Porfiro B, Quante AS, Ramser J, Reis RM, Revillion F, Rhiem K, Riboli B, Ritter J, Rivera D, Rofes P, Rump A, Salinas M, Sánchez de Abajo AM, Schmidt G,

- Schoenwiese U, Seggewiß J, Solanes A, Steinemann D, Stiller M, Stoppa-Lyonnet D, Sullivan KJ, Susman R, Sutter C, Tavtigian SV, Teo SH, Teulé A, Thomassen M, Tibiletti MG, Tischkowitz M, Tognazzo S, Toland AE, Tornero E, Törngren T, Torres-Esquius S, Toss A, Trainer AH, Tucker KM, van Asperen CJ, van Mackelenbergh MT, Varesco L, Vargas-Parra G, Varon R, Vega A, Velasco Ángela, Vesper A-S, Viel A, Vreeswijk MPG, Wagner SA, Waha A, Walker LC, Walters RJ, Wang-Gohrke S, Weber BHF, Weichert W, Wieland K, Wiesmüller L, Witzel I, Wöckel A, Woodward ER, Zachariae S, Zampiga V, Zeder-Göb C, Lázaro C, De Nicolo A, Radice P, Engel C, Schmutzler RK, Goldgar DE, Spurdle AB, KConFab Investigators. Large scale multifactorial likelihood quantitative analysis of BRCA1 and BRCA2 variants: an enigma resource to support clinical variant classification. *Hum Mutat* 2019;40:1557–78.
- 17 Thompson BA, Spurdle AB, Plazzer J-P, Greenblatt MS, Akagi K, Al-Mulla F, Bapat B, Bernstein I, Capellá G, den Dunnen JT, du Sart D, Fabre A, Farrell MP, Farrington SM, Frayling IM, Frebourg T, Goldgar DE, Heinen CD, Holinski-Feder E, Kohonen-Corish M, Robinson KL, Leung SY, Martins A, Moller P, Morak M, Nystrom M, Peltomäki P, Pineda M, Qi M, Ramesar R, Rasmussen LJ, Royer-Pokora B, Scott RJ, Sijmons R, Tavtigian SV, Tops CM, Weber T, Wijnen J, Woods MO, Macrae F, Genuardi M. Application of a 5-tiered scheme for standardized classification of 2,360 unique mismatch repair gene variants in the insight locus-specific database. *Nat Genet* 2014;46:107–15.
  - 18 Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehman HL, ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and genomics and the association for molecular pathology. *Genet Med* 2015;17:405–24.
  - 19 ClinGen variant curation expert panel. ClinGen TP53 expert panel specifications to the ACMG/AMP variant interpretation guidelines version 1, 2019. Available: [https://clinicalgenome.org/site/assets/files/3876/clingen\\_tp53\\_acmg\\_specifications\\_v1.pdf](https://clinicalgenome.org/site/assets/files/3876/clingen_tp53_acmg_specifications_v1.pdf) 2019
  - 20 Brich SE, Abou Tayoun AN, Couch FJ, Cutting GR, Greenblatt MS, Heinen CD, Kanavy DM, Luo X, McNulty SM, Starita LM, Tavtigian SV, Wright MW, Harrison SM, Biesecker LG, Berg JS, Clinical Genome Resource Sequence Variant Interpretation Working Group. Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework. *Genome Med* 2019;12:3.
  - 21 ClinGen variant curation expert panel. ClinGen CDH1 expert panel specifications to the ACMG/AMP variant interpretation guidelines version 2, 2019. Available: [https://clinicalgenome.org/site/assets/files/3982/clingen\\_cdh1\\_acmg\\_specifications\\_v2.pdf](https://clinicalgenome.org/site/assets/files/3982/clingen_cdh1_acmg_specifications_v2.pdf)
  - 22 Mester JL, Ghosh R, Pesaran T, Huether R, Karam R, Hruska KS, Costa HA, Lachlan K, Ngeow J, Barnholtz-Sloan J, Sesock K, Hernandez F, Zhang L, Milko L, Plon SE, Hegde M, Eng C. Gene-Specific criteria for PTEN variant curation: recommendations from the ClinGen PTEN expert panel. *Hum Mutat* 2018;39:1581–92.
  - 23 Tavtigian SV, Greenblatt MS, Harrison SM, Nussbaum RL, Prabhu SA, Boucher KM, Biesecker LG, ClinGen Sequence Variant Interpretation Working Group (ClinGen SVI). Modeling the ACMG/AMP variant classification guidelines as a Bayesian classification framework. *Genet Med* 2018;20:1054–60.
  - 24 Ellard S, Baple EL, Berry I, Forrester N, Turnbull C, Owens M, Eccles DM, Abbs S, Scott R, Deans Z, Lester T, Campbell J, Newman W, McMullan D. ACGS best practice guidelines for variant classification in rare disease 2020: Association for Clinical Genomic Science (ACGS), 2020. Available: <https://www.acgs.uk.com/quality/best-practice-guidelines/#VariantGuidelines>
  - 25 Luheshi L, Hall A, Baple E, Ellard S, McMullan D, Raza, S. Variant classification and interpretation workshop report, 2017. Association for clinical genomic science (ACGS) and PHG Foundation. Available: <https://www.phgfoundation.org/documents/Variant%20classification%20and%20identification%20June%202017.pdf>
  - 26 Turnbull C, Scott RH, Thomas E, Jones L, Murugaesu N, Pretty FB, Halai D, Baple E, Craig C, Hamblin A, Henderson S, Patch C, O'Neill A, Devereau A, Smith K, Martin AR, Sosinsky A, McDonagh EM, Sultana R, Mueller M, Smedley D, Toms A, Dinh L, Fowler T, Bale M, Hubbard T, Rendon A, Hill S, Caulfield MJ, 100 000 Genomes Project. The 100 000 Genomes Project: bringing whole genome sequencing to the NHS. *BMJ* 2018;361:k1687.
  - 27 NHS Genomic Medicine Service. NHS England, 2019. Available: <https://www.england.nhs.uk/genomics/nhs-genomic-med-service/>
  - 28 Cox JH. Available: <https://www.openpseudonymiser.org/About.aspx>

## Appendix 1: CanVIG-UK Consensus Specification for Cancer Susceptibility Genes of ACGS Best Practice Guidelines for Variant Classification (v1.2 03/03/20)

### Guidance notes:

- For the following evidence items, CanVIG-UK have no additional specification for CSGs to add beyond that provided in ACGS Best Practice Guidelines for Variant Classification 2020<sup>1</sup>: PVS1, PS1, PS2, PM4, PM5, PM6, PP1, BS4, BP3, BP7 (**shaded white**).
- For the remaining evidence items, whilst remaining consistent with ACGS Best Practice Guidelines for Variant Classification 2020<sup>1</sup>, there are more specific recommendations pertaining to CSGs contained within the CanVIG-UK Consensus Specification (**shaded grey**).
- A number of disease-specific expert panels have been established by the USA ClinGen Sequence Variant Interpretation (SVI) Working Group, generating disease/gene specific variant interpretation guidelines. Following evaluation within the CanVIG-UK group, in subsequent updates to the CanVIG-UK specification, we shall include specific recommendations regarding adoption and implementation of these disease/gene specific-guidance.

<b>PVS1</b>	<b>_VSTR</b>	<b>_STR</b>	<b>_MOD</b>	<b>_SUP</b>
<i>Null variant (nonsense, frameshift, canonical <math>\pm 1</math> or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where LOF is a known mechanism of disease</i>				
See LOF decision tree and criteria (Tayoun <i>et al</i> 2018) <sup>2</sup>				

<b>PS1</b>	<b>_STR</b>	<b>_MOD</b>	<b>_SUP</b>
<i>Same amino acid change as a previously established pathogenic variant, regardless of nucleotide change</i>			
Use at <b>Strong</b> for a missense variant under evaluation whereby there is a reference missense variant classified as (likely) pathogenic that results in the same amino acid change			
Use at <b>Moderate</b> for an initiation codon variant under evaluation whereby there is a reference variant in the initiation codon classified as (likely) pathogenic			
Use at <b>Supporting</b> for a donor/acceptor splice region variant under evaluation whereby there is a reference variant at the same base residue classified as (likely) pathogenic. The variant under evaluation must be predicted on <i>in silico</i> tools to be equally or more deleterious than the reference variant			

<b>PS2, PM6</b>	<b>_VSTR</b>	<b>_STR</b>	<b>_MOD</b>	<b>_SUP</b>
<b>PS2:</b> <i>De novo (both maternity and paternity confirmed) in a patient with the disease and no family history</i>				
<b>PM6:</b> <i>Assumed de novo, but without confirmation of paternity and maternity</i>				
See ClinGen Sequence Variant Interpretation Recommendation for <i>de novo</i> Criteria (PS2/PM6) <sup>3</sup> <a href="https://www.clinicalgenome.org/site/assets/files/3461/svi_proposal_for_de_novo_criteria_v1_0.pdf">https://www.clinicalgenome.org/site/assets/files/3461/svi_proposal_for_de_novo_criteria_v1_0.pdf</a> .				

PS3

\_VSTR

\_STR

\_MOD

\_SUP

Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product

For assays of protein function

	Discrimination	Controls	Reproducibility
Strong	relative protein activity assay or functional impact	≥10 'true positive' ≥10 'true-negative'	≥2 laboratories OR results demonstrably reproducible from a single laboratory
Moderate	<25% compared to level for wildtype	≥5 'true positive' ≥5 'true-negative'	
Supporting		≥2 'true positive' ≥2 'true-negative'	single laboratory

For assays of splicing function

<b>Very strong</b>	<b>2 orthogonal assays:</b> exhibiting abnormal transcripts; no evidence of leakiness (see notes 4,8)
<b>Strong</b>	<b>1 assay:</b> exhibiting abnormal transcripts; no evidence of leakiness (see note 8)
<b>Moderate</b>	<b>≥1 assay:</b> exhibiting abnormal transcripts; evidence of leakiness (see note 8)
<b>Supporting</b>	<b>≥1 assay:</b> exhibiting abnormal/alternative transcripts which have been reported as present in normal controls (implying naturally occurring isoforms) (see note 12)
<b>Do not apply</b>	<b>≥1 assay:</b> exhibiting abnormal/alternative transcripts with evidence of extreme leakiness (see note 8)

**Explanatory Notes (all functional assays):**

- This criterion is for variant-specific analyses. Where functional data provides support at the gene rather than variant level (e.g. biochemical analysis), this should typically be incorporated within the phenotypic specificity criterion PP4
- To be adopted by CanVIG-UK, a published assay requires independent review by two CanVIG-UK registered clinical laboratory scientists
- Guidance for more quantitative evaluation of functional assays has recently been published by the Clinical Genome Resource Sequence Variant Interpretation Working Group (Brnich *et al* 2020)<sup>4</sup>. Evaluation of these approaches by CanVIG-UK is underway but additional training is required. We anticipate adoption as best practice of these more quantitative approaches for review of functional assays and will use the pan-CanVIG network to collate 'ratings' per assay

**Explanatory Notes (assays of splicing function):**

1. Experimental data may include quantitative assays (e.g. realtime-PCR, Sanger sequencing with formal quantitation of peak height, tape-station quantification of PCR products, minigene assay, RNAseq using NGS) and semi/non-quantitative assays (e.g. visual evaluation of the relative peak height of Sanger sequencing, gel-based evaluation and visualisation of reverse transcriptase PCR (RT-PCR) products, or analysis for evidence of nonsense mediated decay (e.g. where a SNV in trans with the putative splicing variant appears homozygous on RNA sequencing despite being heterozygous on DNA sequencing, indicating the loss of expression of the transcript containing the putative splicing variant))
2. Laboratory methodology should be appropriately validated: primers must have been tested in ≥5 independent normal control reactions, not necessarily run at the same time (i.e. primers could be validated using 5 normal controls across several runs or runs as a batch on a single run)
3. Assays must be performed in a diagnostically ISO accredited laboratory or recognized research laboratory with which direct consultation can be undertaken. **If evidence is derived from an alternative source (e.g. publication only), downgrade by one level of evidence.** All assays should evidence appropriate validations and controls (see note 2).
4. Combinations of assays deemed orthogonal include (a) two PCR-based assays using different primers (b) ≥2 different platforms e.g. RT-PCR and minigene
5. To attain very strong/strong, the criteria by which the disease mechanism is interpreted as loss of function should be met (as per PVS1 recommendations, Tayoun *et al* (2018)<sup>2</sup>)
6. The exon in question must be present in the biologically relevant transcript
7. Splicing impact must fulfil one of the criteria below, **otherwise downgrade by one level of evidence**
  - a) out of frame + predicted to undergo NMD + removal of >10% of the protein
  - b) in-frame but removal of a critical hotspot (as listed in PM1)
  - c) in-frame but removal of >10% of the protein
8. Although there will inevitably be gene by gene and exon by exon variation regarding the lower limit of % normal transcripts ('leakiness') at which normal protein function is maintained, this information is not always known. In the absence of specific data for a given gene/exon, the following thresholds of 'leakiness' should be applied:
  - **Evidence against leakiness:** ratio for allele of >80:20 (abnormal: normal) ==overall ratio of >40:60 (abnormal: normal)
  - **Evidence of some leakiness:** ratio for allele of >20:80 (abnormal: normal) ==overall ratio of >10:90 (abnormal: normal)
  - **Evidence of extreme leakiness:** ratio for allele of <20:80 (abnormal: normal) ==overall ratio of < 10:90 (abnormal: normal). Typically, abnormal transcript will be visible on gel but present only at extremely low level or not visible by Sanger sequencing

The accuracy of different assays in correctly quantifying ratios of different transcripts will vary and is often poorly quantified. As improved data on the precision of different assays emerges, these



- standards will likely be amended
- 9. For  $\pm 1$  or  $\pm 2$ , PVS1 criteria should be used instead of PS3
  - 10. When PS3 is applied for splicing, PP3 (*in silico* evidence), PM4 (in-frame aberration) and PVS1 (truncating) cannot be applied
  - 11. Although PP3 cannot be applied alongside PS3, the assay results for variants at the intron-exon boundaries should nevertheless be supported by *in silico* predictions (MaxEntScan  $\geq 15\%$  difference **OR** SSFL  $\geq 5\%$  difference), **otherwise downgrade by one level of evidence**. Exceptions where *in silico* concordance is not required: (i) U12 splice sites, (ii) TCCTTAAC at the 3' end, (iii) variants outside of intron-exon boundaries (namely 5': Last 3 bases of exon plus 8 bases on intron 3':12 bases of intron plus 2 bases of exon)
  - 12. Naturally occurring (i.e. non-pathogenic) splice variants have been catalogued by expert groups (for example ENIGMA at <https://enigmaconsortium.org/library/general-documents/enigma-classification-criteria/>)<sup>5</sup> and in reference transcriptome resources (for example GTEX, <https://gtexportal.org/home/>)<sup>6</sup>

PS4

\_VSTR

\_STR

\_MOD

\_SUP

The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls

<b>Vstrong</b>	$P_{\text{exact}} \leq 0.0025$
<b>Strong</b>	$P_{\text{exact}} \leq 0.05$
<b>Mod</b>	$P_{\text{exact}} \leq 0.1$
<b>Sup</b>	$P_{\text{exact}} \leq 0.2$

	Cases	Controls	Total
Variant	a	b	a+b
WT	c	d	c+d
Total	a+c	b+d	a+b+c+d

**Explanatory Notes:**

- The  $P_{\text{exact}}$  is generated from Fishers exact 2-way case control comparison (ad/bc)
- Non-duplicated, robustly genotyped case data and control data from equivalent ethnic groups are required
- For Western European case data, comparison to the gnomAD NFE population is recommended (i.e. 64,603 NFE individuals for gnomAD v2.1)
- Where there is no count for the variant in gnomAD v2.1, it is not currently possible to ascertain the correct frequency for the wildtype allele. It is recommended that frequency is inferred from inspection at a nearby base at which a variant has been called. If this is not possible, we recommend using a denominator of 90% of the population size (ie 90% x 64,603 NFE individuals, ie 58,143 individuals) to approximate for the frequency at that base accounting for failed calls
- The p-value does not reflect effect size. Therefore, the Odds Ratio (OR) from this case control comparison (ad/bc) should be consistent with the effect size anticipated for that gene type
  - For a 'high penetrance' gene or variant, OR >4 for unselected cancer series or OR>8 for enriched familial cases
  - For an 'intermediate penetrance' gene or reduced penetrance variant in high penetrance gene, OR >2 for unselected cancer series or OR>4 for enriched familial cases
- If the control frequency is 0, the Haldane-Anscombe correction is required to generate an OR (add 0.5 to cells a,b,c,d)
- If there is uncertainty regarding duplicates in the case series, a commensurately more stringent p-value should be applied
- For non-coding variants, restriction to the WGS partition of gnomAD is required
- Where paired numerator-denominator frequencies are unavailable, a case-counting approach is required, which takes into account the specificity of phenotype observed in the proband +/- family. This approach does not take into account the denominator of the reference case series. For *TP53* and *PTEN*, case-counting guidance has been issued via the respective ClinGen expert groups<sup>7</sup>
- <sup>8</sup>. For MMR, a case-counting approach is under development by CanVIG-UK
- For other rare syndrome cancer susceptibility genes, the UK-ACGS rare disease guidance can be applied. Namely: PS4 can be used at a moderate level of evidence if the variant has been previously identified in multiple (two or more) unrelated affected probands/families with a pathognomonic spectrum of disease, or at a supporting level of evidence if previously identified

in one unrelated affected individual, and has not been reported in gnomAD (in a matched ethnic group)

**PM1, PP2****\_MOD****\_SUP**

**PM1:** Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation

**PP2:** Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease

Use PM1 for missense variants arising in a CSG domain well characterised as a “hotspot” for pathogenic missense variants

Use PM1 at **Moderate** for a variant in a mutational hotspot at which there is no benign variation

Use PM1 at **Supporting** for a variant in a mutational hotspot at which there is some benign variation

Use PP2 at **Supporting** where there is overall constraint for missense variation at the level of the region/exon/gene ( $Z \geq 3.09$ )

**Explanatory Notes:**

- The majority of CSGs act by loss of function. Hence, for many of these genes, the majority of established pathogenic variants are truncating (early linkage analyses, agnostic to mechanism, support this). Examples: *BRCA1*, *BRCA2*, *PALB2*, *RAD51C*, *RAD51D*, *MLH1*, *MSH2*, *MSH6*, *PMS2*. However, in these genes, there are typically specific domains in which missense variation at key residues can cause loss of function. Where benign variation typically also occur in these regions, PM1\_sup can be used. e.g. residues listed in by ENIGMA within *BRCA1* (BRCT and RING domains) and *BRCA2* (DNA binding domain) ([https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA\\_Rules\\_2017-06-29-v2.5.1.pdf](https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf))<sup>5</sup>

**PM2****\_MOD****\_SUP**

*Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium*

Use at **Moderate** where 0 observations of the variant in control series >50,000 individuals

Use at **Supporting** where 1 observation on the variant in control series >50,000 individuals

**Explanatory Notes:**

- Ensure that sequencing coverage is sufficient
- Be cautious in using this criterion for small insertions/deletions, as sequencing approaches/analytical methodologies can result in wide variation in calling of these variant types in NGS/exome/genome data
- If PS4 has been used for case control data
  - The same dataset cannot be re-used for PM2
  - An alternative dataset may be used for PM2 of >50,000 individuals of the same ethnicity as that applied for PS4 **OR**
  - An alternative dataset may be used for PM2 of >50,000 individuals of ethnicity(ies) different to those for PS4, e.g. If the gnomAD NFE has been used for PS4, the remainder of the GNOMAD populations may be used for PM2 (eg 76,853 individuals non NFE from gnomAD v2.1, if NFE used for PS4)
- If PS4 case control data has NOT been used
  - A dataset >50,000 individuals of the same ethnicity as your reference case/family **must** be used for PM2

**PM3****\_STR****\_MOD****\_SUP**

*For recessive disorders, detected in trans with a pathogenic variant*

Use where variant found in trans with a pathogenic variant and the patient-level clinical features match those anticipated for the gene in question

Use at **Strong** where variant found in  $\geq 2$  unrelated cases, and the features are distinctive for that gene

Use at **Moderate** where variant found in 1 case, and the features are distinctive for that gene

Use at **Supporting** where variant found in 1 case, and the features are distinctive for a set of genes

**Explanatory Notes:**

- Comprehensive analysis should be undertaken for the gene to exclude an alternative second pathogenic variant (e.g. including MLPA) in that gene
- Comprehensive analysis should be undertaken for all other genes for which the phenotypic features overlap
- Requires testing of parents (or offspring) to confirm phase
- Can use for homozygous variants but downgrade by one evidence level (as per ClinGen SVI points-based system)<sup>9</sup>

**PM4****\_MOD****\_SUP**

*Protein length changes as a result of in-frame deletions/insertions in a non-repeat region or stop-loss variants*

Use at **Moderate** for

- In-frame insertions/deletions for which PVS1 is not applicable (Tayoun et al 2018<sup>2</sup>)

Use at **Supporting** for

- In-frame insertions/deletions of a single amino-acid

**PM5****\_MOD****\_SUP**

*Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before*

Use at **Moderate** if

- Reference variant is classified as pathogenic [OR likely pathogenic and reported in >1 individual]

Use at **Supporting** if

- Reference variant is classified likely pathogenic and only reported in 1 individual

PM6: see above (PS2)

**PP1****\_STR****\_MOD****\_SUP**

Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease

- See Jarvik and Browning (2016)<sup>10</sup>

PP2: see above (PM1)

**PP3****\_SUP**

*Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact)*

- Protein impact: Using a **predefined** strategy of
  - 3/3 tools (one tool may be marginally below threshold)
    - SIFT (deleterious), Polyphen HumVar ≥ (probably damaging) plus:
      - Align GVGD (C45, C65), (for *BRCA1*, *BRCA2*) **OR**
      - MAPP (bad) (for MMR genes) **OR**
      - CADD ( >15) (for any other CSG)
  - Or use Revel (>0.7) as a single score
- Splicing impact:
  - Intron-exon boundary: MaxEnt >15% difference **AND** SSFL >5% difference<sup>11</sup>
  - Deep intronic: predicted creation of a novel splice site of any strength, absent in the normal sequence

PP5	_SUP
<p><i>Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation</i></p> <ul style="list-style-type: none"> <li>Any classification of LP/P after 2016 from             <ul style="list-style-type: none"> <li>≥2 accredited North American diagnostic laboratories OR</li> <li>a single North American diagnostic laboratory where the utilised evidence is clearly cited</li> <li>an approved ClinGen Expert Group (3 star on ClinVar), e.g. INSIGHT, ENIGMA</li> </ul> </li> <li>When a single laboratory has classified as LP/P with provision of insufficient detail, it is advised that the individual laboratory is contacted to procure directly the evidence used for classification</li> </ul>	



**Additional comments:**

- This is an **exceptional** application, as per UK-ACGS specification. For widely tested cancer susceptibility genes, classifications by large laboratories may have been derived from their substantial series of case data not otherwise publicly available

**BA1/BS1****\_SA****\_STR**

*Allele frequency is “too high” for disorder (ExAC or GnomAD)*

Use **BA1** as **Stand Alone** when allele frequency in a large dataset of heterogeneous outbred population (>10,000 individuals) is: >1% or >0.5% (*BRCA1*, *BRCA2*, *MLH1*, *MSH2*)

Use **BS1** as **Strong** when allele frequency in a heterogeneous outbred population is > value specified for specific gene by respective expert group

**Explanatory Notes:**

- Occasional pathogenic founder mutations occurring at an appreciable frequency in Western Europeans were identified in early characterisation of autosomal dominant CSGs (e.g. *CHEK2* 1100delC at ~0.6%). However, large volumes of sequencing in Western Europeans have now been performed for routinely tested CSGs. The reduction of the threshold of BA1 from 5% to 1% for CSGs and to 0.5% for very well characterised CSGs is predicated on existence of sufficiently high volumes of sequencing data to preclude the existence of hitherto undescribed common founder mutations

**BS2/BP2****\_STR****\_SUP**

**BS2:** Observation in controls inconsistent with disease penetrance. Observed in a healthy adult individual for a **recessive (homozygous)**, dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age

**BP2:** Observed **in trans** with a pathogenic variant for a fully penetrant dominant gene/disorder or observed **in cis** with a pathogenic variant in any inheritance pattern

Use BP2 or BS2 at **Supporting** where no further genotyping or clinical/cellular phenotyping is possible

Use BS2 at **Strong** where

- laboratory analysis has been repeated using an orthogonal approach (e.g. different primers) to confirm homozygosity for allele AND
- patient is of age at which biallelic pathogenic variants would be anticipated to be penetrant for a distinctive phenotype AND
- patient has been actively examined to exclude relevant phenotype AND/OR had analysis of cellular phenotype

OR the homozygote is observed in a specified control population in addition to a heterozygote frequency meeting BS1

Use BP2 at **Strong** where

- alleles have been confirmed as in trans AND
- patient is of age at which biallelic pathogenic variants would be anticipated to be penetrant for a distinctive phenotype AND
- patient has been actively examined to exclude relevant phenotype AND/OR had analysis of cellular phenotype

**Explanatory Notes:**

- BS2 should only be used in the recessive context and for observation of a **homozygote**
- BP2 is used for where the variant is reported as a **compound heterozygote** in conjunction with a pathogenic variant in unaffected individual
- For cancer susceptibility genes, **BP2 and BS2** should only be used for those genes in which typical (non-hypomorphic) biallelic variants cause a recognised phenotype that is fully penetrant from infancy. Such genes include *BRCA2*, *PALB2*, *MLH1*, *MSH2*, *MSH6*, *PMS2*

BS3

\_STR

\_SUP

Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing

**For assays of protein function**

	Discrimination	Controls	Reproducibility
Strong	relative protein activity assay or functional impact	≥10 'true positive' ≥10 'true-negative'	≥2 laboratories OR Results demonstrated as reproducible in single laboratory
Sup	>25% compared to level for wildtype	≥2 'true positive' ≥2 'true-negative'	single laboratory

**For assays of splicing function**

Strong	<b>1 assay:</b> with no evidence of abnormal transcripts (% normal transcript>90%)	ISO accredited laboratory or recognized research laboratory with which direct consultation can be undertaken
Sup	<b>1 assay:</b> with no evidence of abnormal transcripts (% normal transcript>90%)	alternative source of evidence (e.g. publication)

**Explanatory Notes:**

- BS3 should only be applied for an assay of protein function whereby the assay has been validated for variants in the relevant domain to ensure that the mechanism of pathogenicity captured by the assay in question is relevant to that variant
- BS3 should not be applied for an assay of protein function when *in silico* tools predict effect on splicing and/or for the first or last three bases of the exon
- A splicing assay can only be used for BS3 for intronic variants and those in the first or last three bases of the exon
- Evidence of amplification of both alleles is required (i.e. sequencing should demonstrate the SNV in question or another nearby SNV, on the background of the wildtype sequence). This is necessary to exclude generation of a 'normal' RNA result when the splicing aberration has not been detected by the assay used (e.g. due to intron retention, size too large for the PCR to amplify)
- When BS3 is applied for splicing, BP4 (*in silico* evidence), **cannot** be applied. For assays of protein function BS3 and BP4 can be combined.
- For specification of acceptable assays and QC standards, see PS3

BS4		_STR
<i>Non segregation with disease</i>		
<ul style="list-style-type: none"> <li>• See Jarvik and Browning (2016)<sup>10</sup></li> <li>• For cancer susceptibility genes for which the phenotype is non-specific and/or feature age-related/reduced penetrance, phenocopies or hypomorphic variants, expert review is recommended for application of BS4 pertaining to non-segregation.</li> </ul>		

BP1		_SUP
<i>Missense variant in a gene for which primarily truncating variants are known to cause disease</i>		
Use at <b>Supporting</b> for genes/gene regions in which >95% of reported pathogenic variants are truncating		
<b>Explanatory note:</b>		
<ul style="list-style-type: none"> <li>○ Can be used outside of BRCT and RING domains for <i>BRCA1</i> and outside of DNA-binding domain for <i>BRCA2</i>. Other examples of genes for which criterion can be used include <i>PALB2</i> and <i>APC</i></li> <li>○ Variant should be evaluated to exclude splicing impact</li> <li>○ Should not be used if the specific amino acid residue is highly conserved in mammals</li> </ul>		

BP2: see above (BS2)

<b>BP3</b>	<a href="#">_SUP</a>
<i>In-frame deletions in a repetitive region without a known function</i>	
<ul style="list-style-type: none"> <li>Particularly relevant to poorly conserved regions</li> </ul>	
<b>BP4</b>	<a href="#">_SUP</a>
<i>Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)</i>	
<ul style="list-style-type: none"> <li>Splicing impact:             <ul style="list-style-type: none"> <li>Intron-exon boundary: Minimal difference in readings for each of MaxEnt AND SSFL</li> <li>AND no evidence of prediction of exonic/deep intronic novel splice site of any strength</li> </ul> </li> </ul> <b>AND</b> <ul style="list-style-type: none"> <li>Protein impact: Using a <b>predefined</b> strategy of             <ul style="list-style-type: none"> <li>3/3 tools (one tool may be marginally above threshold)                 <ul style="list-style-type: none"> <li>SIFT (tolerated), Polyphen HumVar (benign) plus:                     <ul style="list-style-type: none"> <li>Align GVGD (C0, C15), (for BRCA1, BRCA2)</li> <li>MAPP (good) (for MMR genes)</li> <li>CADD (&lt;10) (for any other CSG)</li> </ul> </li> </ul> </li> <li>Or Revel (&lt;0.4) as a single score</li> </ul> </li> </ul>	
<b>BP5</b>	<a href="#">_SUP</a>
<i>Variant found in a case with an alternate molecular basis for disease</i>	
<b>This should not be applied</b> for autosomal dominant incompletely penetrant non-syndromic genes associated with common cancers e.g. HBOC (hereditary breast and ovarian cancer)	
<b>Explanatory note:</b> <ul style="list-style-type: none"> <li>Co-occurrence of <math>\geq 2</math> pathogenic variants in different cancer susceptibility genes is widely reported. Typically, the phenotype exhibited is indistinguishable from that of a single pathogenic variant</li> </ul>	
<b>BP6</b>	<a href="#">_SUP</a>
<i>Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation</i>	
<b>Explanatory Notes:</b> <ul style="list-style-type: none"> <li>Any classification of LB/B after 2016 from             <ul style="list-style-type: none"> <li><math>\geq 2</math> accredited North American diagnostic laboratory OR</li> <li>a single North American diagnostic laboratory where the utilised evidence is clearly listed</li> <li>ClinGen Expert Group, e.g. INSIGHT, ENIGMA</li> </ul> </li> <li>When a single laboratory has classified as LB/B with provision of insufficient detail, it is advised that the individual laboratory is contacted to procure directly the evidence used for classification</li> </ul>	
<b>Additional comments:</b> This is an <b>exceptional</b> application, as per UK-ACGS specification, as for commonly tested cancer susceptibility genes, classifications by large laboratories may have been derived from their substantial series of case data not otherwise publicly available	
<b>BP7</b>	<a href="#">_SUP</a>
<i>A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved</i>	
<ul style="list-style-type: none"> <li>Not to be used if any cause for suspicion of an impact on splicing</li> </ul>	

## References

1. Ellard S, Baple EL, Berry I, Forrester N, Turnbull C, Owens M, Eccles DM, Abbs S, Scott R, Deans Z, Lester T, Campbell J, Newman W, McMullan D. ACGS Best Practice Guidelines for Variant Classification 2020: Association for Clinical Genetics Science (ACGS), 2020.
2. Abou Tayoun AN, Pesaran T, DiStefano MT, Oza A, Rehm HL, Biesecker LG, Harrison SM. Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion. *Human mutation* 2018;39(11):1517-24. doi: 10.1002/humu.23626 [published Online First: 2018/09/08]
3. ClinGen. ClinGen Sequence Variant Interpretation Recommendation for de novo Criteria (PS2/PM6) version 1.0-, 2018.
4. Brnich SE, Abou Tayoun AN, Couch FJ, Cutting GR, Greenblatt MS, Heinen CD, Kanavy DM, Luo X, McNulty SM, Starita LM, Tavtigian SV, Wright MW, Harrison SM, Biesecker LG, Berg JS. Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework. *Genome medicine* 2019;12(1):3. doi: 10.1186/s13073-019-0690-2 [published Online First: 2020/01/02]
5. Spurdle A. ENIGMA BRCA1/2 Gene Variant Classification Criteria 2017 [Available from: [https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA\\_Rules\\_2017-06-29-v2.5.1.pdf](https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf)].
6. The Genotype-Tissue Expression (GTEx) project. *Nature genetics* 2013;45(6):580-5. doi: 10.1038/ng.2653 [published Online First: 2013/05/30]
7. Mester JL, Ghosh R, Pesaran T, Huether R, Karam R, Hruska KS, Costa HA, Lachlan K, Ngeow J, Barnholtz-Sloan J, Sesock K, Hernandez F, Zhang L, Milko L, Plon SE, Hegde M, Eng C. Gene-specific criteria for PTEN variant curation: Recommendations from the ClinGen PTEN Expert Panel. *Human mutation* 2018;39(11):1581-92. doi: 10.1002/humu.23636 [published Online First: 2018/10/13]
8. Savage SA. TP53 Rule Specifications for the ACMG/AMP Variant Curation Guidelines clinicalgenome.org: ClinGen; 2019 [Available from: [https://clinicalgenome.org/site/assets/files/3876/clingen\\_tp53\\_acmg\\_specifications\\_v1.pdf2019](https://clinicalgenome.org/site/assets/files/3876/clingen_tp53_acmg_specifications_v1.pdf2019)].
9. ClinGen. SVI Recommendation for in trans Criterion (PM3) - Version 1.0, 2019.
10. Jarvik GP, Browning BL. Consideration of Cosegregation in the Pathogenicity Classification of Genomic Variants. *American journal of human genetics* 2016;98(6):1077-81. doi: 10.1016/j.ajhg.2016.04.003 [published Online First: 2016/05/31]
11. Houdayer C, Caux-Moncoutier V, Krieger S, Barrois M, Bonnet F, Bourdon V, Bronner M, Buisson M, Coulet F, Gaildrat P, Lefol C, Leone M, Mazoyer S, Muller D, Remenieras A, Revillion F, Rouleau E, Sokolowska J, Vert JP, Lidereau R, Soubrier F, Sobol H, Sevenet N, Bressac-de Paillerets B, Hardouin A, Tosi M, Sinilnikova OM, Stoppa-Lyonnet D. Guidelines for splicing analysis in molecular diagnosis derived from a set of 327 combined in silico/in vitro studies on BRCA1 and BRCA2 variants. *Human mutation* 2012;33(8):1228-38. doi: 10.1002/humu.22101 [published Online First: 2012/04/17]
12. ClinGen CDH1 Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2, 2019.



## Appendix 2: Example of CanVIG-UK classification summary report

Variant classification by Can-VIG UK (Cancer Variant Interpretation UK)				
Can-VIG UK is a working group convened on behalf of the UK ACGS (Association of Clinical Genetic Scientists), which includes registered clinical scientists and clinical geneticists representing the following UK Regional molecular diagnostic laboratories: Aberdeen, Belfast, Birmingham, Bristol, Cambridge, Cardiff, Dublin, Exeter, Glasgow, GOSH, Guy's, Leeds, Liverpool (Cheshire & Merseyside), Oxford, Manchester, Newcastle, Nottingham, Sheffield, SW Thames (St George's), Wessex (Salisbury/Southampton), University Hospitals of Leicester.				
<b>Submitter</b>	Dr Clare Turnbull MD PhD FRCP FRCPATH		<b>Date</b>	15/11/19
<b>Gene</b>	BRCA1	<b>Transcript</b>	NM_007294.3 ENST00000357654 LRG_292t1	<b>Variant</b> c.53T>C (p.Met18Thr)
<b>Population data</b>	<p>The variant was observed in 7 independent UK families undergoing clinical diagnostic testing, the denominator of which dataset of clinical testing was 25,773. Case control comparison against ethnically matched population data (7/25,773 in familial cases against 0/64,603 GNOMAD NFE controls <math>p_{\text{exact}} = 0.0015</math>)</p> <p>There are additional reports of this variant in ClinVar (6), BIC (3) LOVD3 (22), UMD(7), DMuDB(7)</p> <p>The variant is absent in the remainder of the GNOMAD populations (76,853 individuals)</p>			
<b>Prediction (based on variant type/location), IN silico tools</b>	<p><b>AlignGVGD class:</b> C45</p> <p><b>SIFT prediction:</b> deleterious</p> <p><b>MAPP prediction:</b> bad</p> <p><b>Polyphen2 HumVar prediction:</b> benign</p> <p><b>CADD scaled score [0-99]:</b> 16.18</p> <p><b>SuSPect score [0-100]:</b> 95</p>			
<b>Functional data</b>	Findlay <i>et al.</i> 2018: Non-functional via saturation editing analysis using haploid <i>BRCA1</i> construct			
<b>Segregation data</b>				
<b>De novo data</b>				
<b>Allelic data (biallelic observations)</b>				
<b>Other classifications</b>	Ambry LP 2018, Gene Dx LP 2018, Counsyl LP 2018 Color LP 2018 Enigma 2019 (multifactorial analysis): pathogenic			
<b>Other</b>				

<b>PATHOGENIC Criteria</b>	<b>Weight (supporting, moderate, strong, very strong)</b>	<b>BENIGN Criteria</b>	<b>Weight (supporting, strong)</b>
PVS1 (null)		BS1/BA1 (controls)	
PS4 (case control)	Very strong	BP4 (in silico)	
PM2 (absent control)	Mod	BP1 (only trunc)	
PP3 (in silico)		BP7 (synonymous)	
PM5 (same residue)		BP3 (in frame, no func)	
PM1 (hot spot)		BS3 (functional assay)	
PP1 (Segregation)		BS4 (non segregation)	
PS3 (functional assay)	Strong	BP2 (biallelic)	
PM3 (biallelic)		BP6 (other databases)	
PP5 (other databases)	Sup	Alternative cause (BP5)	
Specific phenotype (PP4)			
De novo (PM6, PS2)			
<b>Total</b>	1 very strong, 1 strong, 1 mod, 1 sup	<b>Total</b>	
<b>Classification</b>	5-Pathogenic		

### Appendix 3: CanVIG-UK Consensus Guidance for Variants of Reduced Penetrance in High Penetrance Cancer Susceptibility Genes

<ul style="list-style-type: none"> <li>• Variant interpretation and classification should be undertaken using the ACMG framework (with ACGS and CanVIG-UK specifications)</li> <li>• If any of the below criteria are met, the variant should be assigned the relevant ACMG class but with addendum of “reduced penetrance”</li> <li>• The report should reference and recommend the nationally ratified clinical management recommendations for that gene for variants of reduced penetrance</li> <li>• Clinical management recommendations for variants of reduced penetrance for each gene should be established by disease-specific experts</li> </ul>
<p><b>Criterion 1: Down-modification of classic biallelic phenotype</b></p> <p>Abnormal physical AND cellular phenotype associated with biallelic mutations is present but notably milder</p> <p><b>Example:</b> BRCA2-related Fanconi anaemia:</p> <ul style="list-style-type: none"> <li>• Cancer is not penetrant by 5 years <b>AND</b></li> <li>• Congenital abnormalities and physical features are mild <b>AND</b></li> <li>• Incomplete functional abrogation of chromosomal breakage following mitomycin C exposure <b>OR</b> BRCA2-specific assays show only modest depletion of BRCA2 in quantity and/or function</li> </ul>
<p><b>Criterion 2: Well calibrated assay gives intermediate effect</b></p> <ul style="list-style-type: none"> <li>• Highly predictive and well-calibrated published functional assay demonstrate intermediate effect, ie significant impairment of protein function but not at level demonstrated for truncating mutations in gene (e.g. Guidugli et al for BRCA2<sup>13 14</sup>, Findlay et al 2019 for BRCA1<sup>15</sup>)</li> </ul>
<p><b>Criterion 3: Segregation analysis gives lower estimate of penetrance</b></p> <ul style="list-style-type: none"> <li>• Formal genetic epidemiologic analyses demonstrate variant to be associated with disease but of penetrance statistically significantly reduced compared to established estimates eg: BRCA1 c.5096G&gt;A p.Arg1699Gln<sup>16 17</sup></li> </ul>

- ClinGen CDH1 Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2, 2019.
- Guidugli L, Carreira A, Caputo SM, Ehlen A, Galli A, Monteiro AN, Neuhausen SL, Hansen TV, Couch FJ, Vreeswijk MP. Functional assays for analysis of variants of uncertain significance in BRCA2. *Human mutation* 2014;35(2):151-64. doi: 10.1002/humu.22478 [published Online First: 2013/12/11]
- Guidugli L, Shimelis H, Masica DL, Pankratz VS, Lipton GB, Singh N, Hu C, Monteiro ANA, Lindor NM, Goldgar DE, Karchin R, Iversen ES, Couch FJ. Assessment of the Clinical Relevance of BRCA2 Missense Variants by Functional and Computational Approaches. *American journal of human genetics* 2018;102(2):233-48. doi: 10.1016/j.ajhg.2017.12.013 [published Online First: 2018/02/06]
- Findlay GM, Daza RM, Martin B, Zhang MD, Leith AP, Gasperini M, Janizek JD, Huang X, Starita LM, Shendure J. Accurate functional classification of thousands of <em>BRCA1</em> variants with saturation genome editing. *bioRxiv* 2018
- Spurdle AB, Whaley PJ, Thompson B, Feng B, Healey S, Brown MA, Pettigrew C, Van Asperen CJ, Ausems MG, Kattentidt-Mouravieva AA, van den Ouweland AM, Lindblom A, Pigg MH, Schmutzler RK, Engel C, Meindl A, Caputo S, Sinilnikova OM, Lidereau R, Couch FJ, Guidugli L, Hansen T, Thomassen M, Eccles DM, Tucker K, Benitez J, Domchek SM, Toland AE, Van Rensburg EJ, Wappenschmidt B, Borg A, Vreeswijk MP, Goldgar DE. BRCA1 R1699Q variant displaying ambiguous functional

- abrogation confers intermediate breast and ovarian cancer risk. *Journal of medical genetics* 2012;49(8):525-32. doi: 10.1136/jmedgenet-2012-101037 [published Online First: 2012/08/15]
17. Moghadasi S, Meeks HD, Vreeswijk MP, Janssen LA, Borg A, Ehrencrona H, Paulsson-Karlsson Y, Wappenschmidt B, Engel C, Gehrig A, Arnold N, Hansen TVO, Thomassen M, Jensen UB, Kruse TA, Ejlertsen B, Gerdes AM, Pedersen IS, Caputo SM, Couch F, Hallberg EJ, van den Ouweland AM, Collee MJ, Teugels E, Adank MA, van der Luijt RB, Mensenkamp AR, Oosterwijk JC, Blok MJ, Janin N, Claes KB, Tucker K, Viassolo V, Toland AE, Eccles DE, Devilee P, Van Asperen CJ, Spurdle AB, Goldgar DE, Garcia EG. The BRCA1 c. 5096G>A p.Arg1699Gln (R1699Q) intermediate risk variant: breast and ovarian cancer risk estimation and recommendations for clinical management from the ENIGMA consortium. *Journal of medical genetics* 2018;55(1):15-20. doi: 10.1136/jmedgenet-2017-104560 [published Online First: 2017/05/12]

**Supplementary table 1: Variants reclassified to date in CanVIG-UK multidisciplinary meeting; RP: reduced penetrance**

Date of CanVIG-UK review	Gene	Variant	Previous UK clinical classifications: most common (others)	CanVIG-UK consensus classification	Submission to ClinVar
Nov-17	BRCA1	c.53T>C p.Met18Thr	4 (3,5)	5	Y
Dec-17	BRCA2	c.8063T>C p.Leu2688Pro	3 (4,5)	5	Y
Jan-18	BRCA1	c.4096+3A>G	3 (2, 4)	2	Y
Feb-18	BRCA1	c.442-22_442-13del	3 (4,5)	4	Y
Mar-18	MLH1	c.122A>G p.Asp41Gly	3 (4,5)	5	Y
April-18	TP53	c.998G>A p.Arg333His	3 (4)	3	Y
Jun-18	BRCA2	c.9302T>G p.Leu3101Arg	4 (3,5)	5	Y
Jul-18	BRCA1	c.4963T>C p.Ser1655Pro	4 (3,5)	5	Y
Jul-18	BRCA1	c.4964C>T p.Ser1655Phe	4 (3,5)	5	Y
Jul-18	BRCA1	c.5207T>C p.Val1736Ala	3 (4)	5	Y
Aug-18	BRCA1	c.4357+6T>C	3 (4,5)	5	Y
Aug-18	BRCA2	c.8378G>A p.Gly2793Glu	3 (4,5)	5	Y
Sep-18	BRCA2	c.8524C>T p.Arg2842Cys	3 (4,5)	5 RP	Y
Oct-18	MLH1	c.1676T>G p.Leu559Arg	3 (4,5)	4	Y
Nov-18	MSH2	c.1807G>A p.Asp603Asn	4 (3,5)	4	Y
Jan-19	MLH1	c.440G>T p.Gly147Val	3 (4)	3	Y
Mar-19	MLH1	c.794G>A p.Arg265His	3	3	Y
Apr-19	BRCA1	c.5357T>C p.Leu1786Pro	3 (4)	4	Y
Apr-19	MLH1	c.1595G>A p.Gly532Asp	3 (4,5)	4	Y
May-19	APC	c.2497A>C p.Ser833Arg	3 (2)	3	Y
May-19	APC	c.6724A>G p.Ser2242Gly	3 (2)	2	Y
May-19	BMPR1A	c.1328G>A p.Arg443His	3 (4,5)	4	Y
May-19	BRCA1	c.5153-26 A>G	3 (4,5)	3	Y
May-19	BRCA2	c.520C>T p.Arg174Cys	3 (4)	3	Y
Sep-19	TP53	c.799C>T p.Arg267Trp	3 (4,5)	4	Y
Oct-19	TP53	c.1141A>G p.Lys381Glu	3 (4)	3	Y
Oct-19	TP53	c.322G>C p.Gly108Arg	4 (3,5)	3	Y
Oct-19	TP53	c.472 C>T p.Arg158Cys	3	3	Y
Oct-19	TP53	c.794T>A p.Leu265Gln	3 (4,5)	4	Y
Oct-19	TP53	c.560-14_560-13delCT	3 (4,5)	3	Y
Jan-20	TP53	c.453-455delCCC p.Pro153del	3 (4,5)	3	Y